

Identification of high-yielding brown planthopper-resistant aromatic rice lines through multi-trait selection

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Abstract. Thanasi A, Chueakaew C, Kongsila S, Onsaarda E, Toojinda T, Katengam S. 2026. Identification of high-yielding brown planthopper-resistant aromatic rice lines through multi-trait selection. *Biodiversitas* 27 (1): d270134. <https://doi.org/10.13057/biodiv/d270134>. Developing rice cultivars that combine durable resistance to brown planthopper (BPH; *Nilaparvata lugens*) with high and stable grain yield is essential for sustaining productivity in rainfed lowland ecosystems of the Mekong Basin. In Northeastern Thailand, rice cultivation is dominated by KDML105, a premium aromatic variety that is highly susceptible to BPH and increasingly vulnerable to climate-driven pest outbreaks and yield instability. This study evaluated 38 aromatic BPH-resistant (ABPH) rice lines developed through marker-assisted breeding under rainfed lowland conditions during the 2020 wet season. Significant genetic variation was detected among the evaluated lines for agronomic traits and yield components, with moderate to high heritability estimates, indicating substantial potential for effective phenotypic selection under target environments. Grain yield exhibited strong positive associations with key yield-determining traits, including spikelet number per panicle, tiller number, panicle length, harvest index, and spikelet fertility, highlighting their importance as indirect selection criteria under rainfed stress conditions. Multi-trait selection approaches integrating yield performance and resistance traits identified six elite ABPH rice lines that consistently combined superior grain yield with stable BPH resistance. These lines represent promising candidates for subsequent multi-environment trials and participatory on-farm evaluation. Overall, the findings demonstrate that integrating resistance breeding with multi-trait phenotypic selection is an effective strategy to accelerate the development of climate-resilient aromatic rice cultivars, contributing to reduced pesticide dependence, improved yield stability, and enhanced food security in rainfed lowland rice systems.

Keywords: Aromatic rice, genetic variability, rainfed lowland ecosystem, Smith-Hazel index, sustainable rice breeding

INTRODUCTION

Rice (*Oryza sativa* L.) serves as a staple food crop for over half of the global population, playing a central role in the food security and economy of Southeast Asia (Singh and Solanki 2024). In Thailand, rice cultivation occurs across a range of agroecosystems, among which rainfed lowland systems are particularly important, especially in the northeastern region of the Mekong Basin. These ecosystems are characterized by strong seasonal dependence on rainfall, limited irrigation infrastructure, and high environmental heterogeneity. Consequently, rice productivity in these areas is often unstable and increasingly threatened by climate variability, highlighting the need for rice varieties with enhanced adaptability and resilience.

Among biotic constraints, the brown planthopper (BPH; *Nilaparvata lugens* Stål) is one of the most destructive insect pests affecting rice production in Asia. BPH damages rice plants through direct phloem feeding, leading to reduced tillering, lodging, and hopperburn, and indirectly through the transmission of viral diseases such as rice ragged stunt virus and rice grassy stunt virus. Yield losses

caused by BPH outbreaks can be severe, particularly in rainfed ecosystems where plant stress may intensify pest damage (Horgan 2024). Recent studies have shown that increasing temperatures and irregular rainfall patterns associated with climate change further exacerbate BPH population dynamics and outbreak frequency, posing serious risks to rice production sustainability (Liu et al. 2020; Yele et al. 2025).

Aromatic rice varieties represent a distinctive and valuable component of rice genetic diversity due to their superior grain quality, cultural importance, and strong market demand. In Thailand, KDML105 (Thai Hom Mali rice) remains the dominant aromatic variety cultivated in rainfed lowland areas. Despite its premium eating quality and widespread consumer preference, KDML105 is highly susceptible to BPH, leading to frequent yield instability and heavy reliance on chemical insecticides. These challenges underscore the urgent need to develop aromatic rice cultivars that combine durable BPH resistance with stable yield performance under variable rainfed conditions, while maintaining desirable grain quality traits.

Marker-assisted selection (MAS) has been widely adopted to introgress major BPH resistance genes into elite rice backgrounds. Previous studies have successfully incorporated resistance genes such as *Bph3*, *Bph14*, *Bph18*, and *Bph32* into high-quality or widely adapted cultivars, including KDML105 derivatives (Jairin et al. 2017; Vanavichit et al. 2018). While these efforts have markedly improved resistance levels, most MAS-based studies have primarily emphasized resistance per se, with limited integration of yield-related traits or systematic evaluation of trait trade-offs under rainfed field conditions.

Grain yield in rice is a polygenic trait characterized by low to moderate heritability and strong genotype \times environment interactions (Wang et al. 2019). Selection based solely on yield is therefore inefficient, especially at advanced breeding stages where multiple traits must be optimized simultaneously (Sabouri et al. 2008; Akbar et al. 2021). Multi-trait selection indices provide an effective framework to address this challenge by combining correlated agronomic traits into a single selection criterion. Among these, the Smith-Hazel Index (SHI) has been extensively applied across crops to improve selection efficiency by integrating phenotypic and genetic variance-covariance structures (Silva et al. 2018; Anshori et al. 2019; Candido et al. 2020; Alam et al. 2024; Rajesh-Kumar et al. 2025).

Despite the proven utility of both MAS and selection indices, their combined application in aromatic rice breeding for rainfed lowland ecosystems remains limited. In particular, few studies have explicitly integrated MAS-derived BPH-resistant aromatic lines with multi-trait selection frameworks to identify genotypes that simultaneously optimize resistance, yield components, and agronomic performance under rainfed lowland field conditions. Therefore, the objective of this study was to evaluate a set of aromatic BPH-resistant rice lines developed through MAS under rainfed lowland conditions and to apply a multi-trait selection approach to identify elite genotypes

combining high yield potential and durable resistance. By explicitly linking resistance breeding with multi-trait phenotypic optimization, this study aims to provide a practical breeding pathway for sustainable aromatic rice production in climate-vulnerable rainfed ecosystems.

MATERIALS AND METHODS

Plant materials

A total of thirty-eight F₇ aromatic brown planthopper-resistant rice lines (ABPH) were developed through a cross between ‘Homwarin’ and the advanced breeding line UBN03078-101-342-4-141. Homwarin is an aromatic, weakly photoperiod-sensitive variety known for its submergence tolerance and broad adaptation to rainfed lowland environments in northeastern Thailand. The male parent UBN03078-101-342-4-141, is a near-isogenic line (NIL) of ‘KDML105’ harboring *Bph3* and *Bph32*, two major BPH resistance genes located on chromosomes 6 and 4, respectively. Pedigree breeding combined with MAS was used to pyramid multiple target loci, including *Bph3* and *Bph32* (BPH resistance), *Sub1* (submergence tolerance), *badh2* (aroma), *Wx^b* (low amylose content), and *SSIIa-TT* (low gelatinization temperature), following established marker protocols (Kongsila et al. 2017).

In the present study, phenotypic validation under rainfed field conditions was conducted for agronomic performance and grain yield, and resistance to BPH was inferred from the confirmed presence of *Bph3* and *Bph32* in the genetic background. Direct phenotypic validation of BPH resistance, submergence tolerance, and grain quality traits was not repeated here, but has been previously confirmed for these lines under controlled and field conditions (Katengam et al. 2023). Accordingly, the current work focuses on multi-trait agronomic evaluation and selection efficiency rather than re-validation of target genes.

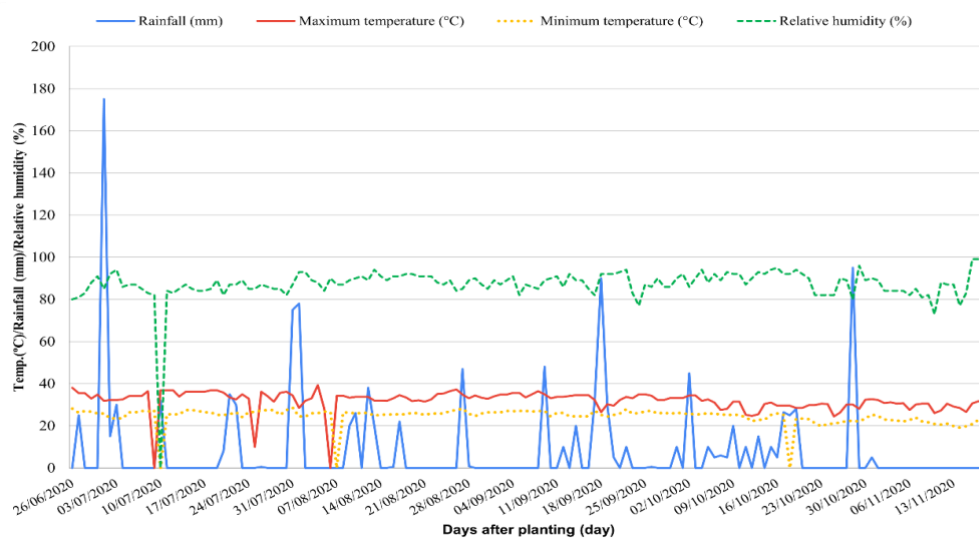


Figure 1. Daily weather data during the 2020 wet season at the experimental site in Ubon Ratchathani University, Thailand. The weather parameter comprised: total daily rainfall (mm), maximum and minimum temperature (°C), and relative humidity (%)

Field experiments

The field evaluation was conducted during the 2020 wet season at the experimental farm of Ubon Ratchathani University, Thailand, located in a representative rainfed lowland rice-growing area of northeastern Thailand. The experimental site is characterized by sandy loam soil with low fertility (organic matter 0.5%, pH 4.9, low available N, P, and K). Weather conditions during the cropping season reflected typical rainfed environments, with high cumulative rainfall, elevated relative humidity, and temperatures ranging from 20 to 40°C (Figure 1).

Thirty-eight ABPH rice lines were evaluated, along with their parental lines (Homwarin and UBN03078-101-342-4-141) and two check cultivars: KDML105 (widely cultivated aromatic variety) and IR57514 (high-yielding, drought-tolerant line). The experiment was arranged in a randomized complete block design (RCBD) with two replications, reflecting practical resource limitations commonly encountered in rainfed field trials. While the limited number of replications reduces inferential power and restricts broad environmental generalization, this design was sufficient to detect genetic differences and identify yield-associated trait relationships under the target rainfed conditions. Such preliminary evaluations are widely used at advanced line screening stages prior to multi-location testing. Seedlings were transplanted at a spacing of 25 × 25 cm. Fertilizer was applied at a total rate of 75-50-75 kg N-P₂O₅-K₂O ha⁻¹ and split into three applications: 40% at the seedling establishment stage, 40% at the tillering stage, and the remaining 20% at the booting stage. Standard rainfed lowland rice management practices were followed throughout the growing season.

Statistical analysis

All statistical analyses were conducted to evaluate genetic variability, trait relationships, and multi-trait selection efficiency. Analysis of variance (ANOVA) was performed to test genotypic effects using the STAR software (IRRI). Mean comparisons were conducted using Tukey's honestly significant difference (HSD) test at $p \leq 0.01$.

Variance components, including genotypic (σ^2_g), environmental (σ^2_e), and phenotypic (σ^2_p) variances, were estimated from ANOVA mean squares, and broad-sense heritability (h^2_B) was calculated as the ratio of σ^2_g to σ^2_p . Pearson correlation coefficients among traits were computed to assess trait associations. Principal component analysis (PCA) was performed to identify major sources of variation and key traits contributing to yield differentiation.

Multi-trait selection was conducted using the Smith-Hazel Index (SHI), integrating grain yield and associated yield components based on their genetic variances and covariances. Correlation analysis, PCA, and selection index calculations were implemented in R (v4.3.2) using relevant packages: corplot, FactoMineR, factoextra, selection.index, and metan. (Kassambara and Mundt 2020; Olivoto and Lúcio 2020).

RESULTS AND DISCUSSION

Genetic variability and heritability of agronomic traits

The analysis of variance indicated highly significant differences among genotypes for all evaluated agronomic traits and grain yield ($p \leq 0.01$), indicating substantial genetic variability within the ABPH rice population (Table 1). Replication effects were not significant for any trait, indicating relatively uniform experimental conditions under the rainfed lowland environment (Table 2).

Broad-sense heritability (h^2_B) estimates ranged from moderate to high across traits, with particularly high values observed for plant height (93.63%), thousand-grain weight (88.00%), and grain yield (92.31%). These results suggest that genetic factors contributed substantially to phenotypic expression of these traits under the specific environmental conditions of the study, consistent with previous reports in rice and other cereal crops (Wang et al. 2019; Kumar et al. 2023). Nevertheless, it is important to emphasize that heritability values are specific to the environment, season, and management conditions under which they are estimated (Sandhu et al. 2019; Wang et al. 2019). The current estimates were derived from a single wet-season evaluation under rainfed lowland conditions and therefore reflect environment-specific genetic control. Environmental variance and genotype × environment (G×E) interactions, which are known to be pronounced in rainfed.

Moderate heritability observed for traits such as number of tillers, number of panicles, spikelets per panicle, panicle length, biomass, and harvest index indicate a greater influence of environmental factors and potential G×E effects (Lee et al. 2023). Consequently, selection for these traits may be more effective when integrated into multi-trait or index-based approaches rather than through direct phenotypic selection alone. Overall, the observed genetic variability provides a useful foundation for selection, while highlighting the need for subsequent multi-environment evaluation to confirm trait stability and breeding value.

Agronomic performance of ABPH lines

Grain yield among the 38 aromatic brown planthopper-resistant (ABPH) rice lines evaluated under rainfed wet-season conditions ranged from 2.05 to 4.42 t ha⁻¹, indicating substantial phenotypic variation within the population (Table 3). Several lines achieved grain yields comparable to, or exceeding, those of the reference cultivars included in the trial. Line G5 produced the highest yield, while lines G27, G30, and G32 consistently ranked among the higher-yielding entries. Relative to the check varieties KDML105 and IR57514, the ABPH lines exhibited competitive performance for key yield components, including panicle length, number of spikelets per panicle, percentage of filled grains, and harvest index. These results suggest that favorable yield expression in selected lines was associated with the combined contribution of multiple yield-related traits under the evaluated rainfed conditions.

Table 1. Estimates of genetic parameters for agronomic traits and grain yield in 38 aromatic brown planthopper-resistant (ABPH) rice lines evaluated under rainfed lowland conditions during the 2020 wet season

Traits	Mean±SE	σ^2_p	σ^2_g	σ^2_e	h^2_B (%)
Plant height (cm)	126.50±4.07	716.68	671.02	45.66	93.63
Number of tillers per plant	10.21±0.17	1.59	0.77	0.82	48.43
Number of panicles per plant	9.96±0.16	1.38	0.66	0.72	47.83
Panicle length (cm)	25.76±0.34	2.49	1.48	1.01	59.44
Spikelets per panicle	131.25±2.85	461.84	218.76	243.08	47.37
Filled grains (%)	90.75±0.45	10.42	6.30	4.12	60.46
1000-grain weight (g)	25.74±0.27	3.25	2.86	0.39	88.00
Grain yield (t ha ⁻¹)	3.00±0.08	0.26	0.24	0.02	92.31
Biomass (t ha ⁻¹)	7.62±0.26	2.81	1.32	1.49	46.98
Harvest index	0.28±0.01	0.0028	0.0017	0.0011	60.71

Note: σ^2_p : phenotypic variance; σ^2_g : genotypic variance; σ^2_e : environmental variance; h^2_B : broad-sense heritability

Table 2. Analysis of variance (ANOVA) for yield and related traits among 38 ABPH rice lines evaluated under rainfed lowland conditions in northeastern Thailand during the 2020 wet season

Traits	dF	Mean square		
		Replication	Genotype	Error
Plant height (cm)	41	148.53 ^{ns}	1387.70**	45.66
Number of tillers per plant	41	0.01 ^{ns}	2.35**	0.82
Number of panicles per plant	41	0.10 ^{ns}	2.05**	0.72
Panicle length (cm)	41	1.52 ^{ns}	3.97**	1.01
Spikelets per panicle	41	680.01 ^{ns}	680.61**	243.08
Filled grains (%)	41	0.01 ^{ns}	16.73**	4.12
1000-grain weight (g)	41	8.80**	6.13**	0.39
Grain yield (t ha ⁻¹)	41	0.01 ^{ns}	0.51**	0.02
Biomass (t ha ⁻¹)	41	1.85 ^{ns}	4.13**	1.48
Harvest index	41	0.005 ^{ns}	0.004**	0.001

Note: **: significance at $p \leq 0.01$; ns: non-significance; dF: degree of freedom

The observed agronomic performance is consistent with the genetic background of the parental materials used in the breeding program. Homwarin, which served as the female parent, was developed through marker-assisted backcross breeding using IR57514 as the source for agronomic adaptation and stress tolerance, with KDML105 as the donor of aromatic grain quality and soft cooking texture. As a result, Homwarin retains key adaptive traits derived from IR57514 while incorporating grain quality attributes from KDML105. The competitive yield performance and associated agronomic traits observed in several ABPH lines, therefore, likely reflect the inheritance and recombination of these parental characteristics, as previously reported for IR57514 and Homwarin under stress-prone lowland environments (Romyen et al. 1998; Wade et al. 1999; Katengam et al. 2011).

Days to flowering among the ABPH lines ranged from 104 to 129 days after transplanting, reflecting the photoperiod sensitivity typical of aromatic rice cultivated in northeastern Thailand (Supplementary Table 2). A small number of lines (e.g., G2 and G3) exhibited excessive plant height (>200 cm), which may increase lodging susceptibility under high rainfall conditions. Overall, the results demonstrate that the ABPH population contains

lines with favorable agronomic performance under rainfed conditions. However, as these observations are specific to a single environment and season, multi-location and multi-season evaluations will be required to confirm yield stability and assess genotype \times environment interactions.

Trait correlations and selection efficiency

Grain yield showed significant positive correlations with several agronomically important traits, including number of spikelets per panicle, number of tillers per plant, number of panicles per plant, panicle length, percentage of filled grains, and harvest index (Figure 2). These associations are consistent with previous reports in rice under stress-prone and rainfed conditions (Jairin et al. 2009; Javed et al. 2015) and indicate that yield variation within the ABPH population was phenotypically associated with traits related to sink size and assimilate partitioning efficiency. Such coordinated relationships among yield and its component traits are characteristic of rice, where grain yield is determined by the integrated performance of multiple, interrelated traits rather than by a single component acting independently (Anshori et al. 2019).

Table 3. Agronomic performance and yield-related traits of 38 ABPH rice lines, parental lines, and check varieties evaluated under rainfed conditions in northeastern Thailand during the 2020 wet season

Genotypes	DF (DAT)	PH (cm)	NTP	NPP	PL (cm)	NSP	TGW (g)	PFG (%)	PUG (%)	GY (t ha ⁻¹)	BM (t ha ⁻¹)	HI
G1	107.00	191.80	7.85	7.75	27.10	105.50	28.30	92.72	7.29	2.05	7.08	0.22
G2	106.00	206.35	9.35	9.05	27.55	104.50	28.92	85.08	14.93	2.08	11.87	0.16
G3	107.00	201.00	9.10	8.65	27.20	110.00	29.97	88.62	11.39	2.50	9.08	0.22
G4	122.00	120.40	9.95	9.80	25.80	141.00	24.27	91.45	8.55	2.88	7.18	0.28
G5	119.00	114.45	10.40	10.05	25.30	167.00	24.83	91.54	8.47	4.42	7.00	0.33
G6	123.00	118.65	10.10	10.00	25.25	141.00	24.79	92.97	7.04	2.97	6.14	0.33
G7	107.00	111.05	11.50	11.15	26.20	123.00	27.25	90.83	9.18	3.58	6.07	0.32
G8	106.00	103.65	11.60	11.25	24.80	118.00	25.07	90.85	9.16	2.96	6.18	0.30
G9	105.00	113.85	12.25	11.80	25.55	112.50	25.95	93.03	6.98	3.96	6.40	0.33
G10	104.00	114.65	12.00	11.50	26.05	134.00	25.72	92.16	7.84	3.38	6.95	0.33
G11	107.00	108.20	12.25	11.75	25.50	115.50	25.71	91.70	8.30	3.33	6.51	0.32
G12	107.00	117.85	10.00	9.60	24.40	137.00	23.27	89.06	10.95	2.59	6.59	0.27
G13	107.00	116.20	11.00	10.35	23.10	133.00	22.53	88.96	11.05	2.81	6.71	0.30
G14	107.00	122.50	10.00	9.85	25.00	138.50	24.56	85.90	14.11	2.72	6.87	0.26
G15	106.00	122.55	11.40	11.00	23.60	138.50	23.38	86.78	13.23	2.74	7.66	0.26
G16	106.00	123.40	10.85	10.50	24.30	138.50	24.81	91.11	8.90	2.82	7.20	0.29
G17	115.00	103.05	10.40	10.20	24.30	153.50	25.89	86.90	13.10	2.53	6.13	0.30
G18	122.00	157.35	9.05	8.95	23.55	136.00	26.72	89.31	10.70	2.96	8.79	0.22
G19	109.00	120.45	10.10	9.95	25.45	119.50	25.47	92.13	7.88	3.20	8.15	0.28
G20	107.00	112.95	11.20	10.65	26.15	136.50	25.21	89.08	10.92	3.05	7.36	0.27
G21	107.00	120.30	11.20	10.80	26.05	133.50	25.34	87.84	12.17	3.46	7.67	0.31
G22	130.00	110.05	8.15	8.10	22.60	102.50	26.19	93.40	6.61	2.26	6.90	0.24
G23	129.00	120.50	9.20	9.00	23.95	128.50	26.38	93.31	6.69	2.76	8.36	0.25
G24	129.00	114.00	9.75	9.70	24.05	123.50	26.42	92.94	7.06	2.72	7.99	0.26
G25	107.00	116.10	9.60	9.15	26.00	119.50	25.40	90.81	9.20	2.66	7.96	0.26
G26	107.00	107.00	9.40	9.25	26.35	140.50	24.62	92.09	7.91	2.80	6.44	0.30
G27	107.00	123.15	10.55	10.45	27.65	143.00	26.90	94.21	5.80	3.80	8.66	0.31
G28	107.00	115.75	8.90	8.45	25.95	115.00	26.78	90.51	9.49	2.52	6.50	0.29
G29	121.00	101.55	9.70	9.70	23.80	104.50	25.28	91.23	8.77	2.38	7.64	0.24
G30	119.00	142.25	10.05	10.05	28.95	146.50	26.76	92.18	7.83	3.54	9.59	0.27
G31	123.00	123.85	9.15	9.15	25.65	133.50	27.09	93.11	6.90	3.39	7.90	0.28
G32	115.00	124.20	9.85	9.85	25.60	131.50	27.86	93.70	6.31	3.62	8.62	0.29
G33	126.00	121.75	8.70	8.60	24.40	118.00	29.46	92.96	7.05	2.76	7.69	0.26
G34	108.00	104.55	11.90	11.90	24.65	122.00	23.39	93.27	6.74	3.10	5.93	0.34
G35	108.00	111.75	11.50	11.00	24.80	124.50	23.64	88.86	11.15	2.92	6.50	0.31
G36	115.00	116.35	10.35	10.00	26.30	188.50	22.88	81.53	18.48	3.16	5.39	0.37
G37	107.00	106.20	10.40	10.40	26.10	140.00	24.64	92.22	7.79	3.25	5.35	0.36
G38	108.00	116.20	11.45	11.10	24.60	134.00	24.70	91.71	8.29	3.18	6.58	0.33
Homwarin (P1)	104.00	135.47	10.77	10.73	28.39	125.50	25.01	94.14	5.86	3.39	11.77	0.27
UBN03078 (P2)	121.00	173.35	9.55	9.15	25.05	111.50	29.13	84.77	15.24	2.49	10.76	0.17
KDML105 (CK1)	115.00	181.84	8.64	8.59	36.49	146.00	25.39	92.82	7.19	2.71	12.97	0.21
IR57514 (CK2)	91.00	126.65	9.90	9.25	28.50	177.50	25.40	93.84	6.16	3.78	6.95	0.35
<i>F</i> – test	-	**	**	**	**	**	**	**	**	**	**	**
Mean	-	126.27	10.24	9.97	25.5	131.25	25.74	90.75	9.25	3.00	7.46	0.28
HSD _{0.01}	-	28.49	3.82	3.58	4.24	65.73	2.66	8.56	8.56	0.61	5.14	0.13
CV (%)	-	5.49	8.74	8.44	3.97	12.13	3.01	2.21	21.71	4.73	16.42	11.72

Note: **: significance at $P \leq 0.01$; mean: average of 38 ABPH rice lines; HSD: Tukey's Honest Significant Difference; Traits: DF: days to flowering (DAT, days after transplanting); PH: plant height; NTP: number of tillers per plant; NPP: number of panicles per plant; PL: panicle length; NSP: number of spikelets per panicle; TGW: 1000-grain weight; PFG: percentage of filled grains; PUG: percentage of unfilled grains; GY: grain yield; BM: biomass; HI: harvest index; P1: Homwarin (female parent); P2: UBN03078-101-342-4-141 (male parent)

Principal component analysis (PCA) was used to further summarize the multivariate relationships among traits. The first two principal components explained 55.99% of the total phenotypic variation (Table 4; Figure 3). Traits that were positively correlated with grain yield also exhibited relatively high loadings on the first principal component, indicating that they contributed jointly to the primary axis of phenotypic variation among the ABPH lines. In the PCA biplot, genotypes such as G5, G27, and G32 were positioned close to grain yield and its associated traits, reflecting similarity in phenotypic profiles under the evaluated environment. This spatial proximity represents shared trait expression patterns rather than direct causal

relationships. The PCA results, therefore, complement the correlation analysis by illustrating how yield-related traits collectively structure variation within the population, with the first principal component capturing the combined influence of these interrelated agronomic attributes.

Taken together, the correlation and PCA analyses indicate that grain yield variation among the ABPH lines is associated with a consistent combination of yield-related traits, particularly those linked to sink capacity and assimilate allocation. These findings support the application of integrated, multi-trait analytical approaches for phenotypic characterization and early-stage selection in aromatic rice breeding programs targeting rainfed lowland environments.

Table 4. Eigenvalues, trait loadings (eigenvectors), and proportion of phenotypic variance explained by the first five principal components derived from yield and yield-related traits in 38 ABPH rice lines

Traits	PC1	PC2	PC3	PC4	PC5
Days to flowering	-0.34	-0.51	0.50	0.18	0.31
Plant height (cm)	-0.77	0.40	-0.20	-0.21	-0.07
Number of tillers per plant	0.77	0.17	-0.51	0.22	0.09
Number of panicles per plant	0.77	0.08	-0.47	0.24	0.09
Panicle length (cm)	-0.13	0.90	0.09	-0.12	-0.23
Number of spikelets per panicle	0.48	0.34	0.48	-0.54	0.31
Percentage of filled grains (%)	0.06	0.16	0.41	0.82	-0.04
1000-grain weight (g)	-0.72	0.21	0.07	0.26	-0.40
Grain yield (t ha ⁻¹)	0.61	0.61	0.36	0.21	0.13
Biomass (t ha ⁻¹)	-0.60	0.55	-0.16	0.18	0.51
Harvest index	0.82	0.00	0.33	-0.11	-0.38
Eigen value	4.06	2.10	1.44	1.31	0.85
Variance (%)	36.86	19.13	13.08	11.93	7.76
Cumulative variance (%)	36.86	55.99	69.07	81.00	88.75

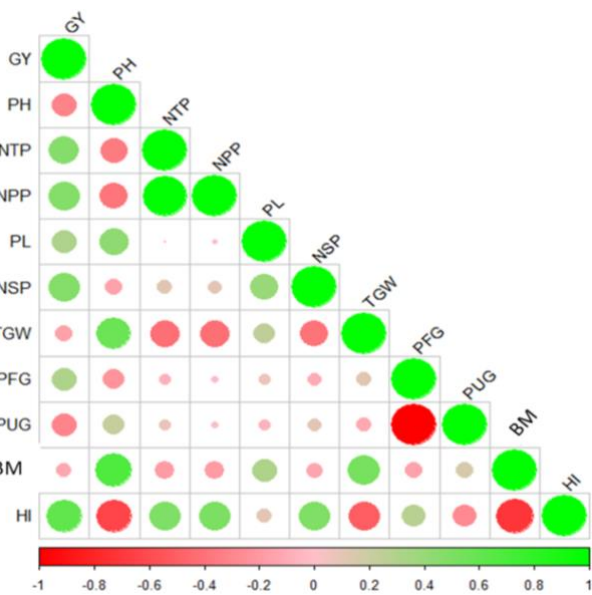


Figure 2. Phenotypic correlation matrix among grain yield and yield-related traits in 38 ABPH rice lines evaluated under rainfed conditions. GY: grain yield, NTP: number of tillers per plant, NPP: number of panicles per plant, PL: panicle length, PFG: percentage of filled grains, PUG: percentage of unfilled grains, BM: biomass, HI: harvest index

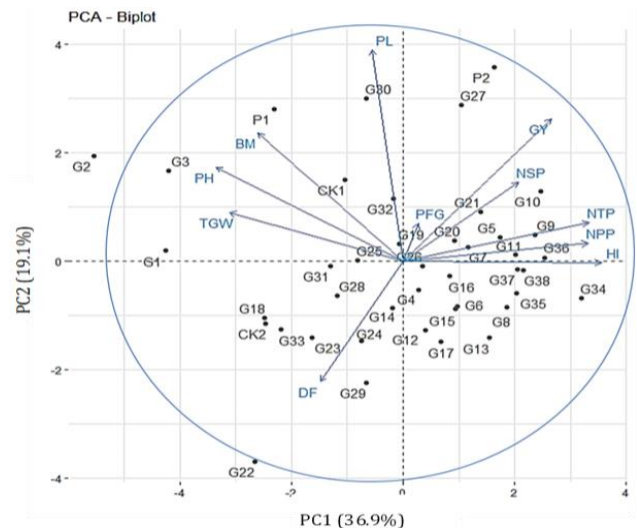


Figure 3. Principal component analysis (PCA) biplot showing relationships among 38 ABPH lines and agronomic traits under rainfed lowland conditions. DF: days to flowering, PH: plant height, NTP: number of tillers per plant, NPP: number of panicles per plant, PL: panicle length, PFG: percentage of filled grains, GY: grain yield, BM: biomass, HI: harvest index

Multi-trait selection using Smith-Hazel Index

The Smith-Hazel Index (SHI) was used to integrate grain yield with multiple agronomic traits that exhibited significant genetic variation, moderate to high heritability, and positive phenotypic associations with grain yield. The traits included in the index—grain yield, number of tillers per plant, number of panicles per plant, number of spikelets per panicle, panicle length, percentage of filled grains, and harvest index—were selected based on evidence from analysis of variance, correlation analysis, and principal component analysis, indicating their collective contribution to phenotypic yield differentiation within the ABPH population. The use of selection indices to combine correlated traits is a well-established approach for improving selection efficiency in breeding programs targeting complex traits such as grain yield (Hadou el hadj et al. 2022).

Economic weights were estimated as standardized partial regression coefficients of grain yield on yield-related traits, aiming to improve grain yield while maintaining balanced agronomic performance under rainfed lowland conditions. In the absence of predefined economic values and where traits exhibit favorable associations with the breeding objective, this method has been widely adopted as a practical and unbiased strategy in early- to advanced-generation selection (Hidalgo-Contreras et al. 2021). Under this configuration, indices that combined grain yield with a greater number of yield-related traits consistently produced higher predicted genetic gain and relative selection efficiency than indices based on fewer traits or grain yield alone (Table 5). This pattern indicates that multi-trait integration captured a broader proportion of yield-associated variation in the population, consistent with theoretical expectations for index selection involving correlated traits (Htwe et al. 2020).

A selection intensity of 20% was applied to identify a practical subset of superior lines for further evaluation. Moderate selection intensities are commonly used in advanced-generation breeding to balance genetic gain with the maintenance of sufficient genetic diversity for subsequent testing stages (Hadou el hadj et al. 2022). Based on SHI values, six lines (G5, G27, G32, G15, G30, and G18) were selected (Figure 4). These lines showed higher mean grain yield than the population average and positive selection differentials for traits related to sink capacity, particularly number of spikelets per panicle and biomass, while smaller or negative differentials were observed for some traits, such as number of tillers per plant and harvest index (Table 6). Such trade-offs among component traits are expected in index-based selection and reflect the redistribution of assimilates among yield components rather than uniform improvement across all traits (Anshori et al. 2019; Guimarães et al. 2021).

From a breeding perspective, the SHI results demonstrate that simultaneous selection for multiple interrelated traits can improve selection efficiency compared with selection based solely on grain yield. This approach is particularly relevant under rainfed lowland conditions, where yield expression is influenced by complex interactions among plant architecture, sink development, and assimilate allocation (Wang et al. 2020). However, because index performance depends on trait expression within a specific environment, the effectiveness of SHI-based selection is inherently environment- and season-specific (Yue et al. 2022). Therefore, multi-location and multi-season evaluations will be required to assess the stability of index rankings, quantify genotype \times environment interactions, and confirm the broader applicability of selected lines before advancement to wider testing or potential release.

Table 5. Predicted genetic gain for grain yield and relative selection efficiency of Smith-Hazel selection indices constructed using different combinations of grain yield (GY) and yield-related agronomic traits in 38 ABPH rice lines evaluated under rainfed conditions

Indices	Genetic advance (t ha ⁻¹)	Relative efficiency (%)
GY+NTP+NPP+NSP+PL+PFG+HI	0.55	188.73
GY+NPP+PL+NSP+PFG+HI	0.54	185.88
GY+NPP+PL+NSP+HI	0.53	182.03
GY+NPP+PL+NSP+PFG	0.49	169.43
GY+PL+NSP+HI	0.52	179.02
GY+PL+NSP+PFG	0.47	163.35
GY+NSP+HI	0.47	162.04
GY+PL+NSP	0.44	152.39
GY+NSP	0.38	131.57
GY+HI	0.36	126.14
GY+PL	0.32	109.34
GY+PFG	0.31	105.82
GY+NPP	0.30	102.38
GY+NTP	0.30	102.34
GY	0.29	100.00

Note: GY: grain yield (t ha⁻¹); NTP: number of tillers per plant; NPP: number of panicles per plant; NSP: number of spikelets per panicle; PL: panicle length (cm); PFG: percentage of filled grains (%); HI: harvest index. Genetic gain is expressed as expected improvement in grain yield (t ha⁻¹) relative to selection based on grain yield alone (100%)

Table 6. Mean performance of grain yield and yield-related traits for the entire population of 38 ABPH rice lines and for six superior lines selected using the Smith-Hazel Index (SHI) at 20% selection intensity

Parameters	Mean of 38 ABPH lines	The mean of 6 selected ABPH lines	Selection differential (%)
Days to flowering	112.42	114.6667	1.99
Plant height (cm)	123.58	130.6583	5.73
Number of tillers per plant	10.27	10.21667	-0.49
Number of panicles per plant	10.01	10.05833	0.46
Panicle length (cm)	25.36	25.775	1.64
Number of spikelets per panicle	130.32	143.75	10.30
Percentage of filled grains (%)	90.69	91.29	0.66
1000-grain weight (g)	25.69	26.08	1.48
Grain yield (t ha ⁻¹)	3.00	3.51	17.30
Biomass (t ha ⁻¹)	7.31	8.38	14.80
Harvest index	0.29	0.28	-2.02

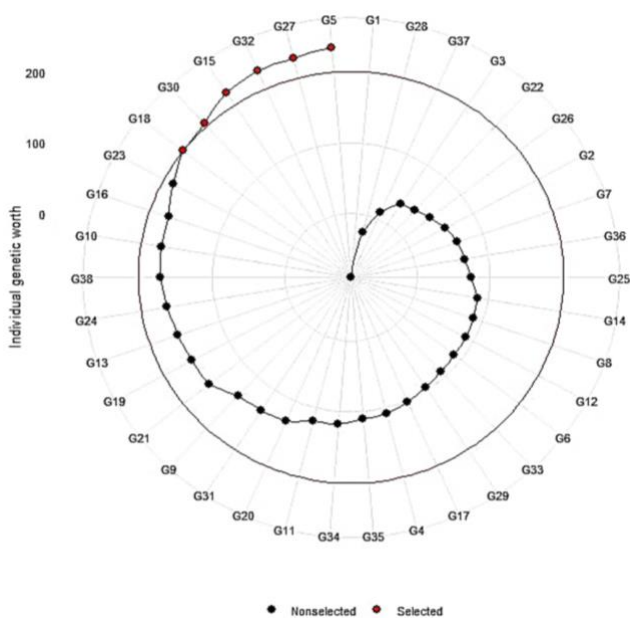


Figure 4. Smith-Hazel Index (SHI) values at 20% selection intensity for 38 aromatic brown planthopper-resistant (ABPH) rice lines evaluated under rainfed conditions. Red symbols indicate six superior genotypes (G5, G27, G32, G15, G30, and G18) selected based on combined performance for grain yield and associated agronomic traits (GY, NTP, NPP, NSP, PL, PFG, and HI), while black symbols represent non-selected genotypes

Implications for climate resilience and regional food security

Rainfed lowland rice systems in the Mekong Basin are increasingly affected by climate variability, including erratic rainfall, intermittent drought and flooding, and intensified insect pest pressure such as brown planthopper (BPH). These interacting stresses threaten yield stability, elevate production risk, and often increase reliance on chemical inputs, undermining the sustainability of rice-based farming systems (Wassmann et al. 2009). Improving varietal resilience through genetic resistance and balanced agronomic performance is therefore a key strategy for

sustaining rice-based food systems under climate change (Wang et al. 2025).

This study demonstrates that aromatic rice lines combining BPH resistance with competitive yield performance can be identified under rainfed conditions through the integration of marker-assisted selection (MAS) and multi-trait phenotypic selection. By retaining the grain quality attributes of KDML105-type aromatic rice while improving resistance and agronomic robustness, the selected lines address a key limitation in regional breeding programs, where premium quality has frequently been associated with vulnerability to biotic and abiotic stresses. Improving the resilience of aromatic rice is particularly important given its economic significance for both household consumption and export markets in Thailand and neighboring countries.

The combined application of MAS and the SHI illustrates a scalable breeding framework for addressing complex trait trade-offs in climate-sensitive rice production systems. By enabling simultaneous improvement of yield, resistance, and associated agronomic traits, this approach supports greater performance stability under rainfed lowland conditions. More broadly, its application can contribute to reduced reliance on chemical pest control and improved production resilience, thereby strengthening climate adaptation and food security strategies in the Mekong Basin and comparable rainfed rice-growing regions.

In conclusion, six elite aromatic rice lines that combine durable BPH resistance with stable yield potential under rainfed lowland conditions have been identified. The integration of MAS with multi-trait phenotypic selection proved effective for capturing favorable combinations of resistance, yield, and associated agronomic traits without compromising grain quality. These findings demonstrate the value of multi-trait breeding strategies for improving aromatic rice adaptation in climate-sensitive environments. Further research should focus on multi-location and multi-season evaluations, including participatory on-farm testing, to assess the stability of performance and genotype \times environment interactions. Such validation is essential before advancement toward varietal release and broader deployment in rainfed lowland rice production systems.

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